Detection of Vitamin A and D in Various Cooking Oils Available in the Market

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ABSTRACT

Vitamin A and D deficiencies are very much widespread in Pakistan. The National Nutrition Survey of 2018 revealed very high rates of these deficiencies. 46% of the pregnant women were deficient in vitamin A and 69% in vitamin D. The problem repeats itself in children under 5, about 54% of children were deficient in vitamin A and 40% were deficient in vitamin D. Vitamin A deficiency creates complications with the vision and enhances infections by reducing immun-ity in adults. Despite getting enough sunlight for most of the year, vitamin D deficiency is also very high and it affects the bone tissues and impairs the proper functioning of bones leading to bone fractures and rickets. To control these deficiencies proper interventions like food fortifi- cation is required. Vegetable cooking oils are consumed at large in Pakistan and are used inalmost all food preparations. The present study attempts to evaluate the various cooking oilsavailable in the markets of Lahore for their vitamin A and D content. AOAC official methods were used for the detection and quantification of vitamins in the oil samples. The antimony trichloride method was used to detect the presence of vitamin A and D. HPLC was used to quan-tify the values of vitamin A and D in 14 cooking oil samples. The results showed that 70% of the edible oil samples were fortified with vitamin A and D but only 28% of them meet the nationally prescribed standards. While 30% of samples were not fortified with vitamin A and D.

Keywords: *Vitamin A, Vitamin D, Food fortification, Oil fortification*

1. INTRODUCTION

Multiple micronutrient deficiencies are prevalent across the globe and can have devastating health consequences. The World Health Organization estimates that more than 2 billion people globally are being affected by micronutrient deficiencies (Ritchie & Roser, 2017). Vitamin deficiencies affectpeople of all genders and ages, as well as certain risk groups. They not only cause specific diseases,but they can also aggravate the infections and the risk for chronic diseases, greatly impacting mor- bidity, mortality, and quality of life (Tulchinsky, 2010).

Deficiencies notably of Vitamin A and D are raging and continue to be significant public health prob- lems, which adversely impact the health of all population groups (Das, Salam, Kumar, & Bhutta, 2013). Deficiencies occur when people do not have access to micronutrient-rich food like fruits, vegetables, meat, andmeat products or the foods that have been fortified with these micronutrients, to overcome their deficiencies because people with limited economical resources cannot afford such expensive forti- fied foods (Thompson & Amoroso, 2011). One of the most cost-effective ways for addressing micronutrient deficiencies and improving out- comes is food fortification (Das et al., 2013). Food fortification is an appealing public health strategy and has the benefit of targeting a wide range of populations at risk without changing their existing consumption pattern (Mary, 2010). Fats and Oils are the indispensable components of our diet due to the presence of essential fatty acids. They are the major source of the energy and also help in the absorption of fat-soluble vita- mins. Since fats and oils are the basis for preparing most diets and they are good solvents for ab- sorbing fat-soluble vitamins hence these are fortified with Vitamin A and D to cope with their defi-ciencies (Diosady & Krishnaswamy, 2018).

In Pakistan, there is severe vitamin and mineral deficiency, particularly of vitamin A and D. 42% of non-pregnant women and 54 % of children under the age of 5 have low Vitamin A levels and 69% ofwomen are vitamin D deficient. The deficiencies of these vitamins affect the immune system and the cognitive abilities of preschool children. Vitamin A and D is a major contributor to maternal newborn and child death rates. Thus, the control and prevention of micronutrient deficiencies can lead to positive health benefits (Gaffey et al., 2014).

Vitamin A and Vitamin D deficiency has become a serious health problem in Pakistan. Fortification of the staple

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food particularly of the cooking oils with these vitamins has become a common inter-vention in eradicating their deficiencies (Rashid & Aslam, 2015).

Edible oil/ghee is consumed by most people daily. In Pakistan, about 100% of households consumeoil/ghee. Therefore, fortifying the edible oil/ghee with vitamin A and D can improve their poor nu-tritional status without changing eating habits after one year of sustained consumption. So, oil for-tified with vitamin A and D can reach vulnerable population groups through this intervention (Balagamwala et al., 2019).

The fortification of edible oil/ghee with vitamin A was authorized under the West Pakistan Pure Food Laws of 1965 and the clause was maintained in all provinces in the Pure Food Law. The existing Pakistan Standard Specification includes the addition of 33,000 (±10%) international units of vitaminA per kilogram of finished product and for vitamin D it is 3750 (±10%) I.U/kg. This standard was issued by Pakistan Standards and Quality Control Authority (PSQCA).

The present study aims to assess the Vitamin A and D content in cooking oils available in the marketsof Lahore. The result of the study can help in making a wise choice regarding the use of cooking oilscommercially or in the household.

2. MATERIALS AND METHODS

A total of 14 cooking oil samples were collected, of different brands from the different supermarkets of Lahore to check their vitamin A and D content. The experimental work was done in the laborato-ries of the Pakistan Council for Scientific and Industrial Research, Lahore. The experiments done were by the AOAC international standards.

2.1. Qualitative Analysis of Edible Cooking Oils

Vitamin A and D content was detected in 14 edible oil samples by the Antimony Trichloride method (Eitenmiller, Landen Jr, & Ye, 2016). The reagent was prepared by dissolving 100g antimony trichloride in 300ml of chloroform. Few drops of reagent were added in 1ml of oil sample the color change was observed when the reagent was reacted with the vitamins present in the oil.

2.2. Quantitative Analysis of Edible Cooking Oils

After the detection of vitamins, 14 edible oil samples were prepared for vitamins A and D studies by standard methods of qualitative and quantitative estimations by chromatography. The sampleswere subjected to the removal of the separation of saponifiable and unsaponifiable edible oils with alkali. The unsaponifiable are solvent extracted, solvents were recovered by rotary thin-film evap- orator. The unsaponifiable were qualitatively identified on TLC and HPLC,

2.3. Saponification

First, the samples were prepared for saponification. 1ml of oil sample was added into 250ml flask and then 300mg of ascorbic acid was added in the same flask. 10ml of aqueous KOH (50%) was added to the flask containing the oil sample. 50ml ethanol was added, and then the flask was sealed by a stopper and shook vigorously. Saponification was performed under the reflux cooler. Successful saponification was indicated by the disappearance of fatty drops.

2.4. Extraction

After saponification, the fat-soluble vitamins were extracted. The saponified solution is transferred quantitatively to a separating funnel through multiple rinsing with water. The sample was rinsed from a flask with 50 ml ethanol into a 250 ml funnel. Distilled water was added (to break emulsion), then extraction was made with ether and hexane in the ratio 3:1 and the vitamins were extracted by shaking on a mechanical shaker for about 10 minutes. The residue was dissolved in 10ml metha- nol.

The extraction solutions were transferred to HPLC vials. And the quantities of vitamin A and D in oilsamples were noted (AOAC 960.29, AOAC 2002.05).

2.5. Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) Version 2.0. Vitamin A and D content in different cooking oils were made by a one-way ANOVA test.

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3. RESULTS

Antimony Trichloride gives blue color in the presence of vitamin A and D. When the drops of anti- mony trichloride were added to oil samples, no blue color was formed in 4 out of 14 oil samples, indicating that they have no added vitamin A and D. The rest of the 10 samples, however, indicatedblue color, some showed a very strong color while others just showed slight color change for about 2 seconds questioning the quantity of these vitamins in the oil samples (Table 1).

Four samples of edible oils were identified as they have not been fortified with vitamins. It was alsofound out that the TLC indicated the presence of vitamins in the remaining ten samples. However, their strengths were very important to meet the prescribed specifications and international stand- ards. The unsaponifiable vitamins were separated and the concentration of vitamins was deter- mined and the values were noted in Table 2.

The results in Figure 1 shows that only four samples (28.6%) contain vitamin A in the range of 30000 - 36000 IU/kg. While six samples (42.8%) have vitamin A in the range of 15000-25000 IU/kg. Vitamins A has not been found in the remaining four samples. The statistical analysis indicated that only 14%of samples contain the requisite contents of vitamin D prescribed by national standards, 29% in therange of 1500-3000 IU/kg while in the 57% of the samples, the oil manufactures attempted to add fat-soluble vitamins but it is a matter of question that their limits are not up to the mark *(*Figure 2*)*.The one-way ANOVA test was applied to the vitamin A content of 14 cooking oil samples, the test results interpreted that there was a highly significant difference between mean vitamin A content in 14 different brands. This shows that there lies a significant difference in vitamin A content among 14 samples (Table 3) The one-way ANOVA test was also applied to the vitamin D content of 14 cook- ing oil samples, the test results interpreted that there was a highly significant difference between mean vitamin D content in 14 different brands. This shows that there lies a significant difference invitamin D content among 14 samples (Table 4).

Table 1. Qualitative analysis of Vitamin A and D in 14 edible oil samples.

Sr No.	Sample ID	Vitamin A&D
1	A	D
2	B	ND
3	C	D
4	D	D
5	E	D
6	F	D
7	G	D
8	H	D
9	I	ND
10	J	D
11	K	D
12	L	ND
13	M	D
14	N	ND

ND→ Not Detected.
D→ Detected.

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Table 2. Quantitative determination of Vitamin A and D in 14 edible oil samples

Sr No.	Sample ID	Vitamin A (IU/Kg)	*Standard Specs (IU/Kg) (Vitamin A) (33,000, ±10%)	Vitamin D (IU/Kg)	*Standard Specs (IU/Kg) (Vitamin D) (3750, ±10%)
1	A	29000	29700-36300	2100	3000-4500
2	B	650	29700-36300	220	3000-4500
3	C	29800	29700-36300	3250	3000-4500
4	D	23500	29700-36300	1250	3000-4500
5	E	31400	29700-36300	3100	3000-4500
6	F	29700	29700-36300	2700	3000-4500
7	G	24800	29700-36300	1800	3000-4500
8	H	20000	29700-36300	900	3000-4500
9	I	1600	29700-36300	800	3000-4500
10	J	9800	29700-36300	950	3000-4500
11	K	10000	29700-36300	1100	3000-4500
12	L	700	29700-36300	400	3000-4500
13	M	25200	29700-36300	2200	3000-4500
14	N	800	29700-36300	530	3000-4500

*PSCQA-Standard Specifications of Vitamin A and D Vitamin D (IU/Kg).

Table 3. One-way Anova test among 14 cooking oil samples

Vitamin A					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	5967581250.000	13	459044711.538	4079.346	.000
Within Groups	3150812.000	28	112529.000		
Total	5970732062.000	41			

Table 4. One-way Anova test among 14 cooking oil samples

Vitamin D					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	39481114.286	13	3037008.791	7006.942	.000
Within Groups	12136.000	28	433.429		
Total	39493250.286	41			

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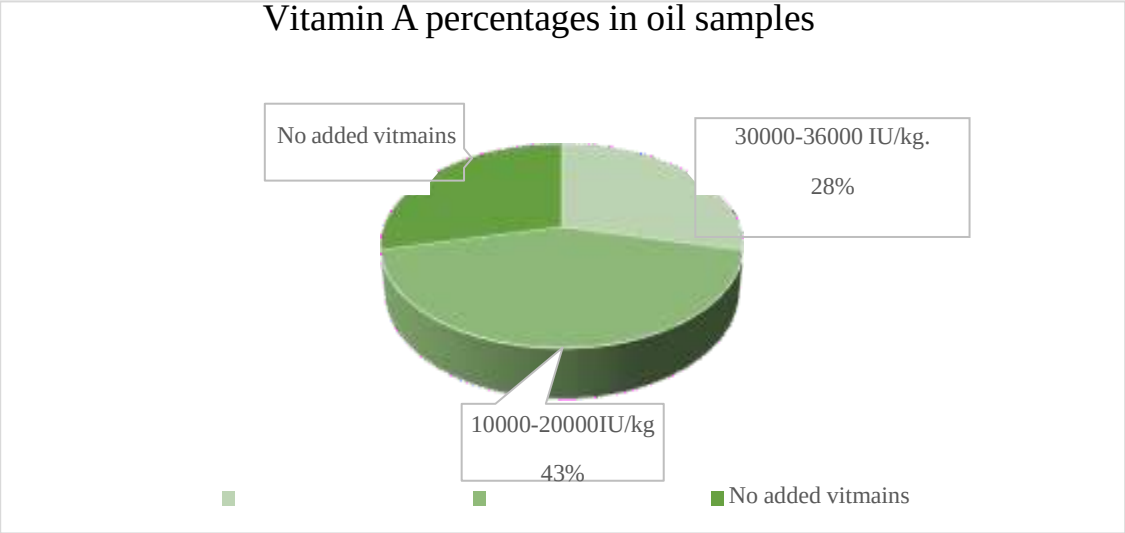

Figure 1. Presence of Vitamin A in oil samples

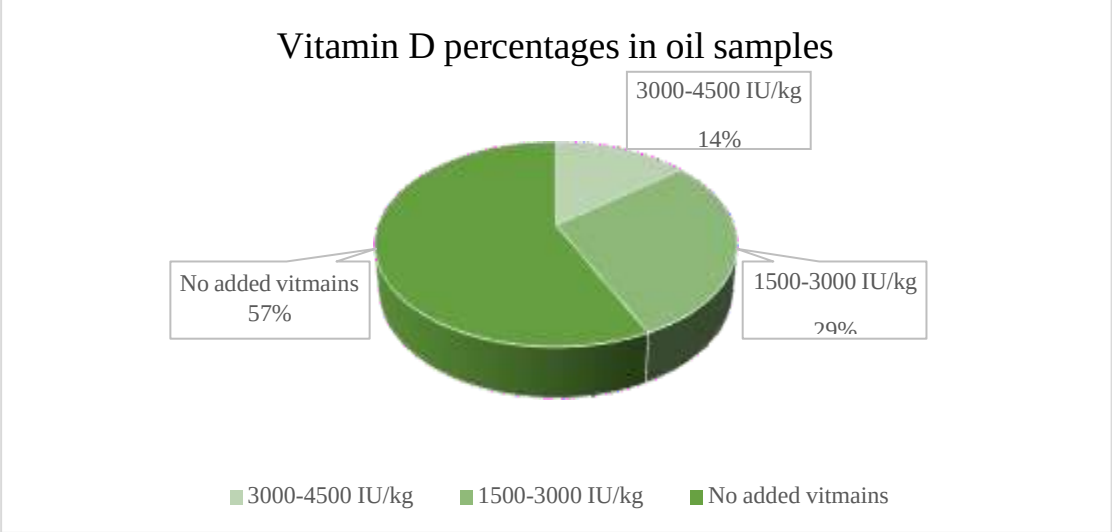

Figure 2. Presence of Vitamin D in oil samples

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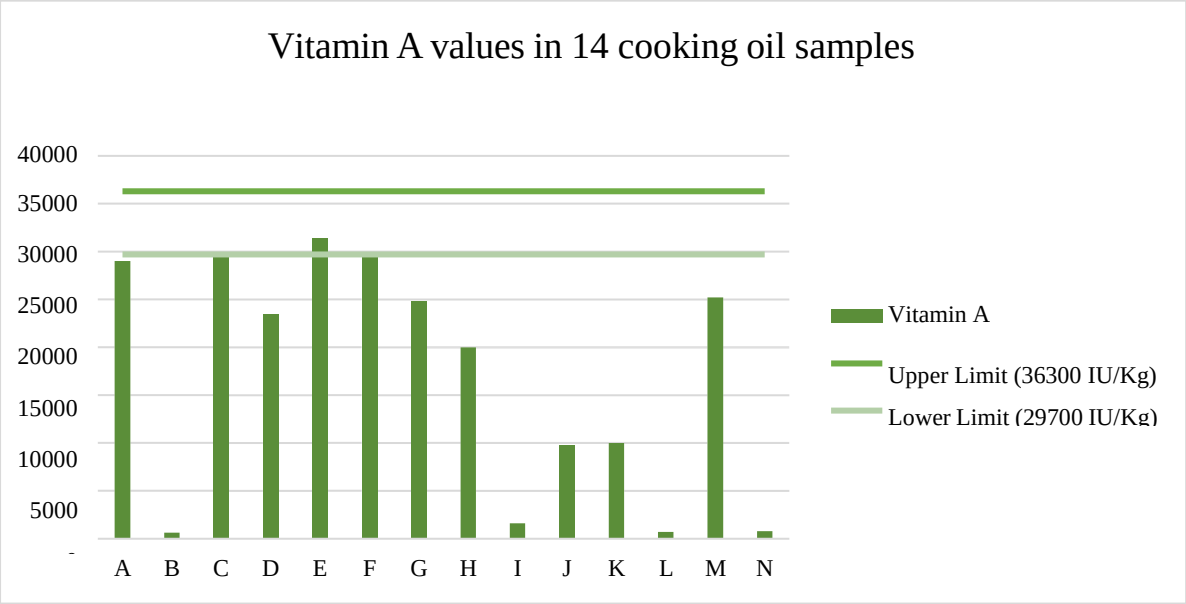

Figure 3. Comparative analysis of vitamin A content in 14 cooking oil samples

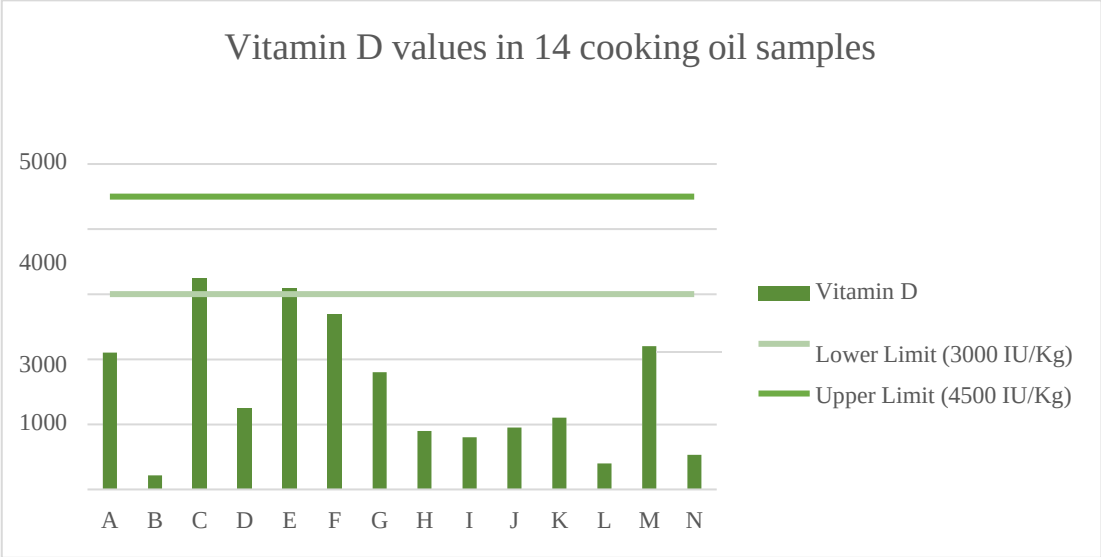

Figure 4. Comparative analysis of vitamin D content in 14 cooking oil samples

4. DISCUSSION

Keeping the account of the above facts, PSQCA recommends the addition of Vitamin A in cooking oils in the range of 29700-36300 IU/ kg and the addition of Vitamin D in the range of 3000-4500 IU/kg. But practically no fortification of these vitamins is in practice markedly because many factors may affect the amount of these vitamins in the oils. Improper processing, no certified labs, un- trained employees all can lead to deterioration of vitamin A and D which are sensitive to oxidation. Moreover, many companies do not check the quality of the vitamins being added in the oils which causes them to break up hence not reaching the prescribed level of fortification.

The previous study by Mehmood *et al*., 2012 reported the same results, that no fortification of vit- amin is in practice only 10 samples out of 35 contained vitamin A. Another study by Jungjohann, Ahmad, Arshad, and Rasool (2017) on-market evaluation of vitamin A oil/ghee fortification in Pakistan concluded the 2/3rd of the oil/ghee does not follow the fortification requirements in which one-third is not fortified. The findings of their study are following the results of this study.

This may be due to many factors that involve the handling and packaging of the fortified edible oils.Fat-soluble vitamins are liable to oxidation so proper processing, handling, and packaging are very important to prevent the

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oxidation of these vitamins in oils (Marc Pignitter et al., 2016). People who addvitamins to the oils usually have no idea about the prescribed limits of these vitamins. They may add vitamins but their quantity is not checked by the higher authorities. Substandard products are also fortified but improper processing can lead to the deterioration of vitamin A and D. Certified labs and eligible employees also play an essential role in maintaining the quality and quantity of these vitamins in the oils. Some companies also don't add vitamins to minimize the cost for their benefits. The type of packaging of oils can also lead to the eradication of these vitamins in the oils due to oxidation. So, all these factors can result in affecting the quality and quantity of vitamins in the oil (M. Pignitter & Somoza, 2017).

5. CONCLUSION

The qualitative results indicated that 30% of the samples were identified that they have not been fortified with vitamin A and D during industrial processing. The remaining 70% of the samples con- tain Vitamin A and D lower or higher contents beyond the limit, the HPLC results were found out. The deviation of results is may be due to negligence during quality control at the plant site, storage, and supply. Vitamin A and D are not present in abundance in foods so they must be taken in the form of supplementation or the foods that are fortified with these vitamins. Fortification is a cost- effective strategy, and fortifying the edible oil can help in combating the deficiencies of vitamin A and D and because the consumption of oil is widespread, so fortified edible oils can reach several affected populations in rural as well as in urban areas.

Although the fortification of edible oils has been in practice since the 1960s, many factors affect thequality of the fortified products and when these products reach the general population with the added vitamins in them are almost deteriorated not reaching the prescribed standards.

6. LIMITATIONS

- The study is confined to 10-14 branded edible oils samples that are commonly available in the mar-kets of Lahore.
- The study is limited to the labs of PCSIR.
- The edible oil samples collected for experimentation were in pouched packaging.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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